PRIORITY DOCUMENT

PCT/DK 97/00368

09/254310

REC'D 30 OCT 1997

WIPO PCT

Kongeriget Danmark

Patent application No.: 0953/96

Date of filing: 04 Sep 1996

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- The specification, claims and drawings as filed with the application on the filing date indicated above.

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TAASTRUP 29 Sep 1997

Gurli Brehmer
Kontorfuldmægtig

A MICROFLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS

FIELD OF THE INVENTION

The present invention relates to methods and apparatuses for detection, separation and sorting of particles, such as cells, molecules, etc in a fluid. In particular, the invention relates to magnetic and/or electrical particle separation, e.g. for utilization in flow cytometry, electrophoretic separation, etc.

BACKGROUND OF THE INVENTION

Flow cytometry is a well known technique that is used for high throughput measurements of optical and/or electrical characteristics of microscopic biological samples. Flow cytometry instruments analyze and isolate cells and organelles with particular physical, biochemical, and immunological properties.

Cell sorting by flow cytometry (fluorescence activated cell sorting) has long been the method of choice for isolation of specific cell populations by surface markers. However, cell sorting by flow cytometry suffers from several drawbacks, especially high dilution of desired cell sample, low speed and sterility problems. Also the equipment is very costly with high running and maintenance cost, making the technique available only to a small number of laboratories.

During the last few years, isolation of cells by antibody-coupled magnetic beads and carriers has been developed into a reliable tool for the isolation and characterization of cell populations. Commercially introduced by Dynal A/S and Miltenyi Biotec immunomagnetic cell separation has become an established method for cell analysis in clinical diagnostic. Its reasonable cheap prize makes it to an alternative to flow cytometry. Especially in sorting of rare cellular event the method is used. For example, sorting of fetal cells in

maternal blood provides a non-invasive alternative to prenatal diagnostic procedures, such as amniocentesis of chorionic villus sampling. Another rare event scenario is the detection of low concentration of cancer cells which has an important role in diagnosis of minimal residual disease and evaluation of appropriate therapies. Another medical application for cell sorting systems is the diagnosis of bacterial and viral diseases.

Although this method offers relatively inexpensive approach to sort rare cellular event, it adds considerable time onto the overall rare event detection and it does not offer the multiparameter analysis readily available with flow cytometry techniques. Existing techniques for magnetic separation are suffering from the low purity of the sorted cell fraction and the low recovery rate of the sorted cells. In most systems several washing steps have to be implemented into the separation procedure which causes cell losses. Additionally small subpopulation of labelled cells cannot be directly isolated by existing magnetic separation techniques.

A good overview about fluorescence activated cell sorting procedures and magnetic activated cell sorting is given in Melamed et. al., "Flow Cytometry and Sorting, (Ed. Melamed et. al., Wiley & Sons Inc., 1990).

SUMMARY OF THE INVENTION

Thus, it is an object of the present invention to provide a method and apparatus for particle separation having an improved efficiency of particle separation compared to the prior art.

It is another object of the present invention to provide a method and apparatus for particle separation in which cell lysis is minimized.

It is yet another object of the present invention to provide an improved method for preparation of samples containing fluids for separation and analysis of particles.

It is a further object of the present invention to provide a method and apparatus for particle separation adapted to be combined with different detection and separation methods and systems.

It is a still further object of the present invention to provide a method and apparatus for simultaneous separation of particles into a plurality of groups of particles.

According to the invention these and other objects are fulfilled by a microflow system for separating particles, comprising a member having a flow channel defined therein for laminar flow of a fluid, first inlet means for entering particles into the flow channel, first outlet means for discharging fluid from the flow channel and field generating means positioned proximate to the flow channel for generating a field substantially across the flow channel whereby particles entering the flow channel and being susceptible to the field generated across the flow channel are deflected in the flow channel.

The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may comprise living cells, chromosomes, organelles, biomolecules, such as proteins, etc, etc.

The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may be magnetically stained to facilitate separation in a magnetic field.

According to an important aspect of the invention, the flow through the flow channel is a laminar flow so that flow of particles are predictable and easy to control, e.g. with a flow of guiding buffers.

The microflow system may comprise second inlet means for entering a first guiding buffer into the flow channel together with the fluid containing particles. When the flow is laminar, the two fluids flow through the flow channel in parallel abutting each other along a small area extending along a longitudinal axis of the flow channel. Particles in the fluid containing particles may then be deflected into the guiding buffer fluid when the two fluids pass the field generated across the flow channel. Further, two outlets may be provided at the down stream end of the flow channel for discharging the guiding buffer now containing separated particles and fluid substantially without particles susceptible to the field generated across the flow channel, correspondingly.

The microflow system may further comprise third inlet means for entering a second guiding buffer for improved control of the path of particle flow through the flow channel. By adjustment of the flow velocities of the guiding buffers and the fluid containing particles, the flow within the flow channel of fluid containing particles may be controlled to flow within a cylinder with a longitudinal axis extending substantially parallel to a longitudinal axis of the flow channel and further the position within the flow channel and the diameter of the flow cylinder may be controlled by corresponding adjustments of the volumetric ratio between the flow rate of the fluid containing particles and the flow rate of the guiding buffers.

When the flow is laminar, the stream of particles can be positioned as desired within the flow channel, e.g. by controlling flow velocities of the fluid containing particles at the particle inlet of the member and flow velocities of guiding buffers at corresponding inlets.

Preferably, the flow channel is small for the flow through the channel to have a low Reynolds number, e.g. below 50 and preferably below 25. Thereby, inertial effects, which causes turbulences and secondary flows are negligible, viscous effects dominates the dynamics, and mixing is caused only by diffusion. Flow of fluid containing particles and guiding buffers can be laminated in guided layers through the channel and displacement of particles in the channel is only caused by the force.

The microflow system may comprise flow speed adjustment means for adjustment of the time the particles reside in the flow channel.

Preferably, the fluid channel is sized so that for efficient separation, particles are displaced 10 - 30 μm in the flow channel. Thereby, the force has only to be exposed to a particle over a very short period of time and thus, continuous separation of particles may be performed.

Preferably, the channel depth is small enough, e.g. below 50 μ, to allow observation of the particles flowing through the channel by a microscope.

The microflow system may comprise a cover, e.g. a transparent cover, for covering the flow channel, the flow channel and the cover defining a flow chamber.

The member with the flow channel may be produced from any suitable material, such as silicon, polymers, such as plexiglass, teflon, etc, glass, ceramics, metals, such as copper, alumina, stainless steel, etc, etc.

The channel may provided in the member by any suitable manufacturing process, such as machining, etching, etc.

In a preferred embodiment of the invention, the member is a silicon chip manufactured utilizing photolithography and the channel is etched into the silicon chip.

The field may be a magnetic field, a electric field, a gravity field, etc, and any combination of such fields.

A magnetic field may be generated by permanent magnets, such as rare earth magnets, such as samarium-germanium magnets, a mixture of ferromagnetic powder and epoxy, etc, etc, electromagnets, etc. The magnets are preferably positioned adjacent to the flow channel so that the magnetic field is substantially perpendicular to a longitudinal axis of the flow channel.

In a preferred embodiment of the invention, the magnets are positioned in and glued to rectangular slots that are etched into a silicon chip.

An electric field may be generated by electrodes, such as metal electrodes, such as gold electrodes, etc, etc. The electrode may be position inside the flow channel, e.g. to introduce electrophoretic forces, e.g. for separation of charged molecules in the fluid, or outside the flow channel e.g. to introduce dielectrophoretic forces, e.g. for separation of particles contained in the flow. Preferably, the electrodes are positioned in such a way that the electric field is substantially perpendicular to a longitudinal axis of the flow channel.

The field generated across the flow channel may be utilized for immobilization of particles whereby particles may be held in substantially fixed positions within the flow channel for a specific period allowing chemical reactions with the particles to take place and/or kinetic measurements on the

particles to be performed and/or to bring the particles into contact with different chemical substances.

The positions in relation to the flow channel of the field generating means may be adjustable for adjustment of the strength of the field across the flow channel.

It is an important advantage of the present invention that a microflow system is provided that operates continuously with no requirement for operator intervention.

It is another advantage of the present invention that separation is performed in one step.

It is still another advantage of the present invention that the particles are separated in a continuous flow without substantially interfering with the flow itself and that separated particles may be collected at corresponding separated outlets of the flow channel without having to interrupt the flow in the flow channel.

It is yet another advantage of the present invention that the microflow system is easily integrated into other continuous flow systems, such as flow cytometers, flow injection analysis systems, etc.

It is a further advantage of the present invention that particles may be separated into a plurality of groups of particles, e.g. different subpopulations of cells, based on different susceptibility to the field generated across the flow channel of the different groups of particles.

It is a still further advantage of the present invention that the micro flow system allows observation of particles in the flow channel using a microscope.

It is yet another advantage of the invention that a closed system is provided allowing biohazardous samples, such as samples containing pahthogenes, to be entered into the system without contaminating the laboratory environment and without causing hazard for operators working with pathogen biomaterials.

It is a still further advantage of the invention that a system with a low shear stress in the flow is provided allowing a gentle treatment of biological samples, e.g. fragile living cells.

According to a second aspect of the invention, a microflow system for analyzing components of a fluid is provided that comprises a member having a flow channel defined therein for laminar flow of a fluid, first inlet means for entering particles into the flow channel, first outlet means for discharging fluid from the flow channel and a plurality of areas located in the flow channel and comprising immobilized reagents so that the fluid is analyzed for a plurality of components in one operation.

The microflow system of the previous section may further comprise field generating means positioned proximate to at least some of the areas adapted to comprise immobilized reagents, each field generating means generating a field proximate to the corresponding area whereby reagents entering the flow channel and being susceptible to the field generated at the area are attracted to and immobilized at the area or are rejected from the area.

According to an important aspect of the invention, a new system for immunomagnetic cell separation and manipulation is provided that utilizes a silicon based microfabricated flow chip. The system combines the advantage of flow cytometry and immunomagnetic separation technique. The flow chip will be a key component of a portable micro system for cell sorting and analysis. The flow chip is designed for rapid immunomagnetic

cell separation without any pressure drop. Its simple and cheap fabrication and versatile sorting and detection properties offers an alternative to conventional cell separation systems.

It is an advantage of the invention that a microflow system is provided that is cheap, easy to operate, versatile, simple and portable and that offers the possibility of automation.

A miniaturized flow channel system is provided that utilizes the advantageous fluid behaviour in microsystems. The invented system operates continuously. Instead of holding back the magnetizable particles in the separation unit, the particles are deflected into the direction of the magnetic field while passing it continuously. By splitting the fluid flow into two or more outlets, the deflection of the particles can be used for separation of particles into different sets of particles, each of which leaves the flow channel through a specific outlet.

The continuous separation system CSS allows efficient enrichment as well as depletion of labelled sample contents of interest. The CSS is designed to fit under a microscope allowing parallel detection of the optical properties of the sample and the control of separation of particles.

An advantage of the geometry of the invented separation flow channel is that a magnetised or electrically charged particle has to be moved only over a distance of 10 - 30 μm to be separated from the fluid containing particles.

Furthermore the invention enables isolation of multiple cell or particle subpopulations from a single sample at the same time. The magnitude and direction of the force F on a magnetizable particle, e.g. a magnetically labelled cell, is dependent on the magnitude of the magnetic field and the number of magnetic moments inducible on a labelled cell.

$F = N*S*\mu B* \text{ grad } B$

where S is the number of Bohr magnetons (μB) per particle and N is the number of particles per cell.

Beads with small S are moving a less distance in lateral direction in relation to the flow through the flow channel than beads with a higher S value. This can be used to separate subpopulation of cells labelled with different magnetizable beads: By splitting the flow channel in various outlet channels cells can be distinguish and separated due to their individual F values.

THE DRAWINGS

Exemplary embodiments of the invention will now be described with reference to the accompanying drawings in which

Fig. 1 illustrates the operation of particle separation according to the present invention,

Fig. 2 shows a cross-sectional view of a separation flow channel according to the present invention,

Fig. 3 shows a flow diagram of a magnetic particle separation apparatus according to the present invention,

Fig. 4 shows flow diagrams of various embodiments of the present invention,

Fig. 5 shows a flow diagram of two flow channels coupled in parallel,

Fig. 6 shows a microflow system with electrodes, and

Fig. 7 shows a flow channel having an array of electrodes.

DETAILED DESCRIPTION OF THE DRAWINGS

According to a preferred embodiment of the invention, magnetically stained particles, e.g. cells labelled immunologically with magnetic particles, such as antibody-coupled magnetic beads, are separated from non-magnetic particles, i.e. non-labelled cells, by exposing the particles to a magnetic field generated with a permanent or an electro magnet. Positive or negative selection methods may be employed. By positive cell separation, cells of a specific cell type are separated and isolated from a heterogenous mixture of cells.

Fig. 1 illustrates the principle of the separation method according to the invention. A microflow system 1 is shown having three inlet and three outlet ports. The fluid 9 containing particles enters the separation flow channel 5 through a central inlet port 2 and is continuously guided through the separation flow channel 5 of the microflow system 1 by two guiding buffers 10 and 11, each of which enters the separation flow channel through inlet ports 3 and 4, correspondingly. A separation magnet 8 is located adjacent to the flow channel 5 and generates a magnetic field across the flow channel 5. When the fluid 9 containing particles passes the magnetic field, magnetically stained cells 12 (particles) are drawn into the guiding buffer 10 and leave the flow channel 5 together with the guiding buffer 10 through the outlet 6 while non-labelled cells 13 which are not influenced by the magnetic force remain in the fluid 9 leaving the flow channel 5 through the waste drain 7.

Due to the small channel dimensions, the flow is laminar with negligible influence of inertia forces. Mixing of sample stream and the guiding buffers is not detectable since the contact area is small and the contact time is reduced to a subsecond range. The thickness of the sample stream can be precisely adjusted by variation of the flow rate of the two guiding buffers. This enables the adjustment and optimization

of the magnetic microflow system for various cell types and sizes.

The magnetic field in the microflow channel operates as an extremely sensitive filter for magnetic particles, e.g. cells. Cells labelled with superparamagnetic beads (e.g. MACS, Dynal) are magnetized and attracted by the magnetic field whereby the flow of magnetized particles is deflected into the separation drain. The short residence time of the fluids in the flow channel and the low Reynold numbers of the flow in the flow channel minimize the influence of gravity compared to the influence of the magnetic force.

Fig. 2 shows a cross-sectional view of the microflow system 1 manufactured utilizing semiconductor technology. The microflow system may be manufactured in any suitable material such as polymers, glass, semiconductors, such as silicium, germanium, gallium arsenide, etc, etc. The microflow system 1 shown is a 3-layer sandwich. The central layer 14 is a silicon wafer having a flow channel 5 etched into it. The silicon wafer 14 is covered with a transparent plate 16, such as a glass plate, having a thickness of, e.g., 0.16 mm. Fluids inside the flow channel 5 may be observed through the glass plate 16, e.g. utilizing a microscope 18. The fluid inlet 2 and outlet 7 are connected to capillary tubings 20, 22 for entering fluids into or discharging fluids from the flow channel 5. The bottom plate 14, e.g. made of plastic, facilitates mounting of the tubings 20, 22.

Characteristic features of an exemplary embodiment of a microflow system according to the invention, e.g. as shown in Figs. 1 and 2, is shown in Table 1.

Table 1 Characteristics, microflow system

Manufacturing method	Material: Silicium Oxide, SiO_2 Photo-lithography Wet-chemical etching
Flow Channel	
Cross sectional area	0.2 - 0.5 mm width x 0.05 - 0.1 mm depth
Length	1.0 - 3.5 mm
total flow rate [µl/min]	1 - 200
flow velocity [mm/min]	15 - 180
Reynold number	0.1 - 20
separation time	0.1 sec [100 µl/min] sec - 2.1 sec [2 µl/min]
Magnet	
Permanent Magnet	
Rare Earth Samarium-Germanium 0.5 x 0.5 x 0.2 mm	
Electro-magnet	
Holding Magnet 25 mm 12 V D.C. RS	

Fig. 3 shows a microflow apparatus 24 including a microflow system 1 as shown in figs. 1 and 2. The microflow system 1 has two inlets 26, 28 and two outlets 30, 32, two syringe pumps 34, 36, two 3-way control valves 38, 40, and capillary tubings 20, 22. The capillary tubings 20, 22 are used for interconnecting the two syringe pumps 34, 36 with the inlets 26, 28 of the microflow system 1.

Conventional syringe pumps which are controlled to generate a predetermined volumetric flow rate have been utilized for generating a continuous flow from the buffer reservoir 44 to the inlet tube 28 and a continuous flow from the sample reservoir 42 to the inlet tube 28.

Fig. 4 illustrates various microflow systems 50, 52, 54, 56 having flow channels of different geometries. Microflow systems with two or three inlet ports and two, three or five outlet ports, respectively, are shown in Fig. 4.

The illustrated flow channels of the microflow system have a width ranging from 0,2 to 0,55 mm, and a depth ranging from 0.04 to 0.1 mm. The system is operating with total volumetric flow rates of 1,0 and 200 µl/min which gives a flow velocity of 15 mm/min up to 100 mm/min. The average residence time of a particle inside the flow channel which corresponds to the separation time ranges from 0,1 to 6 sec. The residence time of the sample is defined by the total volumetric flow rate of the system. The higher the flow rate the lower is the influence of the magnetic field on the sample flow stream. Detailed characteristics are listed in table 1.

The magnets may be positioned in rectangular slots that are etched into the silicon wafer 1. The slots are located adjacent to the separation flow channel 5. As shown in Fig. 1, a permanent magnet 8 or a electro magnet 8 can be received by slots in the microflow system 1. The slots are, e.g., 0.5 mm wide, 0.5 mm long and 0.2 mm deep. For generation of a magnetic field, a solid magnetic block, i.e. rare earth magnet can be glued into the slot. Alternatively, a mixture of ferromagnetic powder and epoxy can be injected into the slots.

The strength of the magnetic field inside the microflow system 1 can be adjusted. If an electro magnet is used for generation of the magnetic field, the magnitude of the field may be varied by varying the amplitude of the voltage input

to the electromagnet. If a permanent magnet generated the magnetic field, the magnitude of the field may be varied by varying the distance between the magnet and the flow channel of the microflow system 1.

The diameter or thickness of the flow layers in the microflow system 1 is adjustable by changing the flow ratio of the sample stream and control buffer stream. Microscopic observation enables a visual control of the flow inside the microflow system 1. A thickness of the sample stream layer less than 1μm may be achieved.

As already mentioned, the net displacement of a particle in the microflow system 1 depends on the force applied to it in the field. This can be utilized for separation of a first group of particles of various types in a fluid into a plurality of set of particles, each set comprising a specific type of particles. A microflow system 1 with e.g. five separation outlets may be used to separate a fluid containing particles into five sets of particles, each set comprising particles that are influenced by the field with a force of a specific magnitude. in the following denoted particles with a specific F-value. Particles with a low F-value are only deflected by a small amount by the field and are discharged from the flow channel through a corresponding outlet port. Particle deflection is increased with increasing F-values whereby such particles are discharged from the flow channel through corresponding other outlets.

Separation speed may be increased by utilization of a plurality of flow channels coupled in parallel as shown in Fig. 5. Fig. 5 shows two flow channels 60, 62 operating in parallel. The fluid containing particles enters the flow channels 60, 62 through inlet ports 64, 66, respectively. The guiding buffer enters the flow channels through the inlet ports 68, 70, respectively. In the flow channels 60, 62, particles are separated from the fluid containing particles by the magnetic fields generated by magnets 72, 74,

respectively, whereby particles susceptible to the magnetic field are deflected from the fluid containing particles into the corresponding guiding buffer and flow with the guiding buffer to the sort outlet 86. Fluid containing particles without the deflected particles leave the flow channels 60, 62 through corresponding outlets 88, 90.

Fig. 6 shows a microflow system 100 utilizing electrodes 102, 104 to generate an electric field across the flow channel 106. The electrodes 102, 104 may introduce dielectrophoretic or electrophoretic forces utilized for particle separation. For electrophoretic separation to take place, gold electrodes may be positioned inside and at the walls of the flow channel 106. By applying a voltage across the electrodes, an electrical field is generated substantially perpendicular to a longitudinal axis of the flow channel. The electrical field cause deflection of charged particles or molecules in the flow channel 106 whereby electrically charged particles can be deflected away from the fluid containing particles flowing in the flow channel and into a guiding buffer also flowing in the flow channel and abutting the fluid containing particles in the flow channel.

Fig. 7 shows a flow channel 110 having a plurality of assay sites, each of which has field generating means that may be individually turned on and turned off. The flow channel 110 shown has rectangular electrodes 112 positioned in small grooves at the bottom of the flow channel 110. A voltage can be applied selectively to each electrode 112. Various probes, receptors, indicators, etc may be attracted to and be immobilized at selected electrodes by applying a voltage to the selected electrodes while a fluid with the corresponding probes, receptors, indicators, etc flows in the flow channel 110. In this way, a plurality of assay sites may be created in the flow channel for simultaneous multicomponent analysis of a sample fluid flowing in the flow channel after immobilization of probes, receptors, indicators, etc.

EXAMPLE 1

A microflow system has been tested utilizing it for separation of various magnetizable particles. The test conditions are listed below.

Particle concentration	10^7 particles/ml
Total flow rate	25 µl/min
Length flow chip	3.5 mm
Separation time	2.4 sec
Desired particle deflection:	10 µm

The separation efficiency (enrichment rate) E and depletion rate 1/E are defined by

$$E = \frac{\frac{\%\ positive\ particles\ after\ separation}{\%\ negative\ particles\ after\ separation}}{\frac{\%\ positive\ particles\ before\ separation}{\%\ negative\ particles\ before\ separation}}$$

For separation of various paramagnetic standard beads of different sizes and paramagnetic field strength, the results are shown in the table 2.

Table 2, separation efficiencies

Paramagnetic Bead	Size	Separation Efficiency [%][1]		
	μ	A)	B)	C)
Polysciences				
25 % iron-oxide	1-10	>99	>99	95
50 % iron-oxide	1-10	>99	>99	96.5
Paesel + Lorei				
Magnetic Affinity	0.5-1.5	>99	>99	97.5
Boehringer				
Streptavidin Magnetic	1	90.5	88.7	89.5
Dynal				
Magnetic Mass Dyna M-450	1-10	98.0	>99	96.5

[1] total flow rates: A)= 10 μl/min, B)= 50 μl/min, C)= 100 μl/min

EXAMPLE 2

Further, the microflow system has been tested utilizing it for separation of Human T-lymphocytes (JURKAT cells). Magnetically stained and un-stained JURKAT cells were used to form a heterogeneous cell sample. For magnetic staining of the cells a CD4-magnetic surface marker from Miltenyi Biotech was used (JURKAT cells were suspended in 1% PBS/BSA to a concentration of 10^7/ml. Biotin-conjugated CD4 magnetic microbeads were added at 2 μl stock/10^7 cells following manufacturers instruction).

The magnetically stained cells (10^7 cells/ml) flowed through the microchip for 10 min and fluids were collected at two outlets. Three experiments at different flow rates (5, 25,

50µl/min) were performed. The same experiments were performed using magnetically unstained cells.

An aliquot of the collected samples were analyzed under a microscope and the particles were counted using a Neubauer microscopy chamber. For each experiment 1 µl sample was analyzed:

Run	flow rate [µl/min]	cells [%] at Sort outlet
negative (unstained cells)		
	5	<0.1
	25	<0.1
	50	<0.1
Control[1]		
	5	n.n.
	25	n.n.
	50	n.n
Positive (stained cells)		
	5	95.5
	25	92.8
	50	80.5
Control[1]		
	5	n.n.
	25	n.n
	50	n.n.

[1] using the microflow system without an integrated magnet

CLAIMS

1. A microflow system for separating particles, comprising a member having a flow channel defined therein adapted for flowing of a fluid containing the particles in the channel, first inlet means for entering particles into the flow channel, first outlet means for discharging fluid from the flow channel and field generating means positioned proximate to the flow channel for generating a field substantially across the flow channel whereby particles entering the flow channel and being susceptible to the field generated across the flow channel are deflected in the flow channel.

2. A microflow system according to claim 1, further comprising second outlet means for discharging particles having been deflected in the flow channel.

3. A microflow system according to claim 1 or 2, wherein the field generating means comprise a magnet.

4. A microflow system according to any of claims 1-3, wherein the field generating means comprise an electrode.

5. A microflow system according to any of the preceding claims, wherein positions in relation to the flow channel of the field generating means are adjustable for adjustment of the strength of the field across the flow channel.

6. A microflow system according to any of the preceding claims, further comprising flow speed adjustment means for adjustment of the time the particles reside in the flow channel.

7. A microflow system according to any of the preceding claims, further comprising a cover for covering the flow channel, the flow channel and the cover defining a flow chamber.

8. A microflow system according to claim 7, wherein the cover is a transparent cover allowing observation of events in the flow channel.

9. A microflow system according to any of the preceding claims, further comprising second inlet means for entering a first guiding buffer for reception of deflected particles.

10. A microflow system according to claim 9, further comprising third inlet means for entering a second guiding buffer for control, by the first and second guiding buffers, of the flow path of fluid containing particles through the flow channel.

11. A microflow system according to claim 10, wherein the width and the position of the flow of fluid containing particles is controlled by adjustment of the volumetric ratio between the particle flow rate and the flow rate of the guiding buffers.

12. A microflow system according to any of the preceding claims, wherein the particles comprise living cells.

13. A microflow system according to any of the preceding claims, wherein the particles comprise chromosomes, organelles, biomolecules, proteins.

14. A microflow system according to any of the preceding claims, wherein the particles have been magnetically stained.

15. A microflow system according to any of the preceding claims, comprising a plurality of outlets for discharging of particles separated according to their different susceptibility to the field across the flow channel.

16. A microflow system for analyzing components of a fluid, comprising a member having a flow channel defined therein for laminar flow of a fluid, first inlet means for entering particles into the flow channel, first outlet means for discharging fluid from the flow channel and a plurality of areas located in the flow channel and comprising immobilized reagents so that the fluid is analyzed for a plurality of components in one operation.

17. A microflow system according to claim 16, further comprising field generating means positioned proximate to at least some of the areas adapted to comprise immobilized reagents, each field generating means generating a field proximate to the corresponding area whereby reagents entering the flow channel and being susceptible to the field generated at the area are attracted to and immobilized at the area or are rejected from the area.

18. A method of separating particles, comprising the steps of

entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, and

generating a field substantially across the flow channel whereby particles flowing through the flow channel and being susceptible to the field generated across the flow channel are deflected in the flow channel.

19. A method of analyzing components of a fluid, comprising the steps of entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, the channel having a plurality of sites, each of which comprises immobilized reagents whereby the fluid can be analyzed for a plurality of components while it is flowing through the channel.

20. A method of forming sites comprising immobilized reagents in a flow channel, the method comprising generating a field proximate to the corresponding site while a reagent is flowing proximate to the site, the reagent being susceptible to the field generated at the site whereby the reagent is attracted to and immobilized at the site.

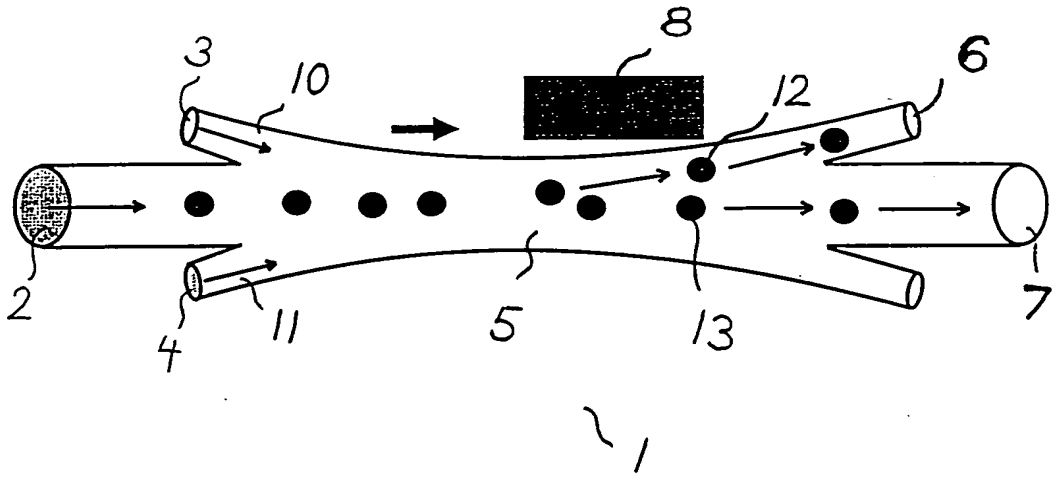

Fig. 1

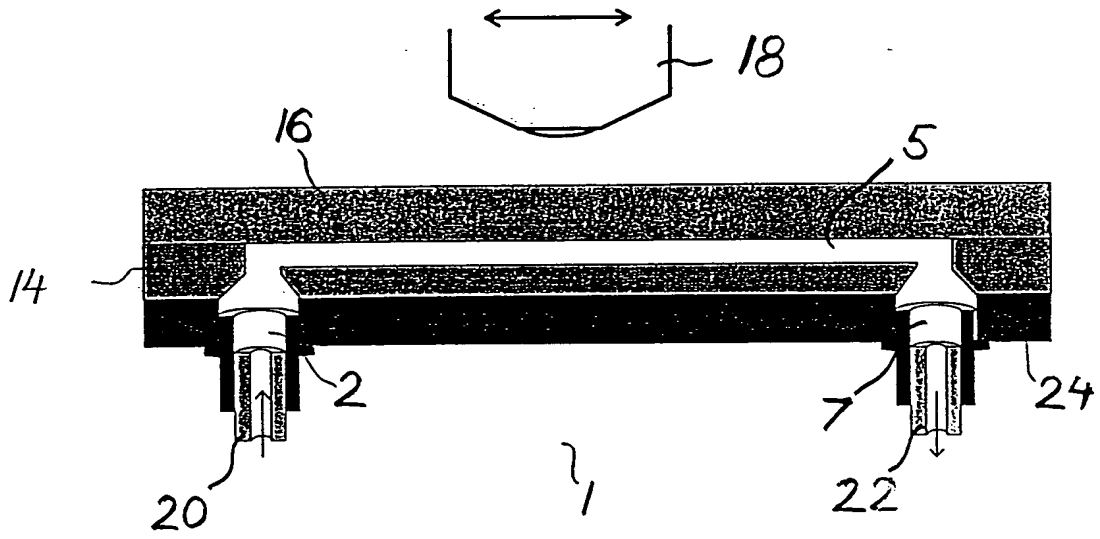

Fig. 2

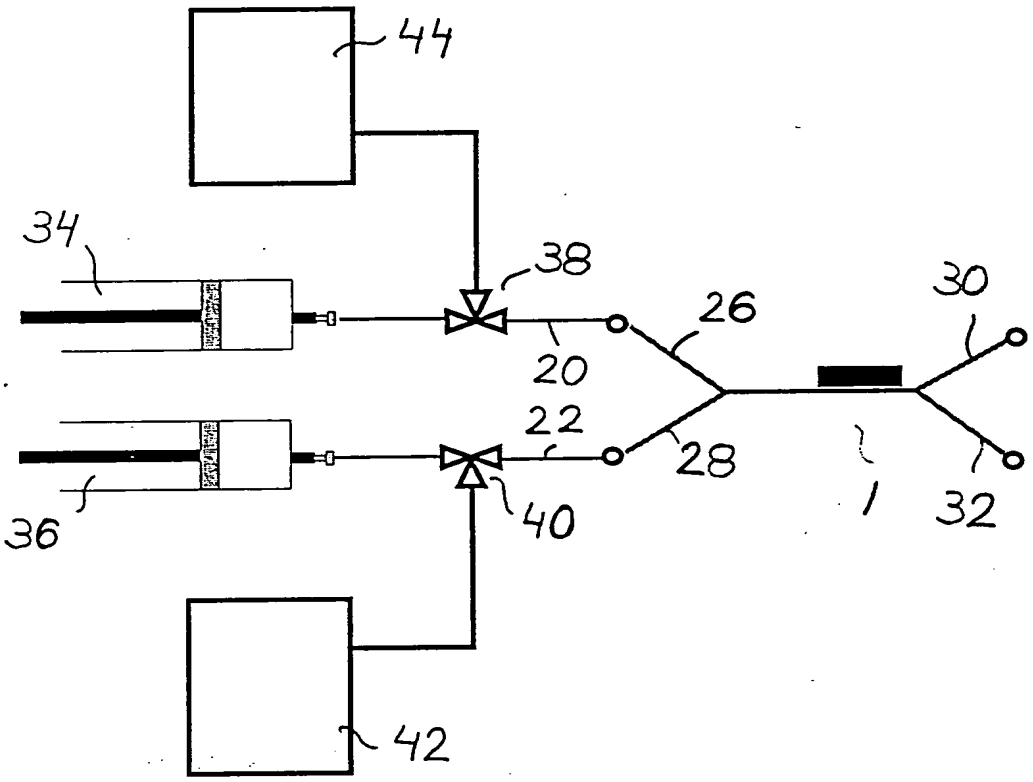

Fig. 3

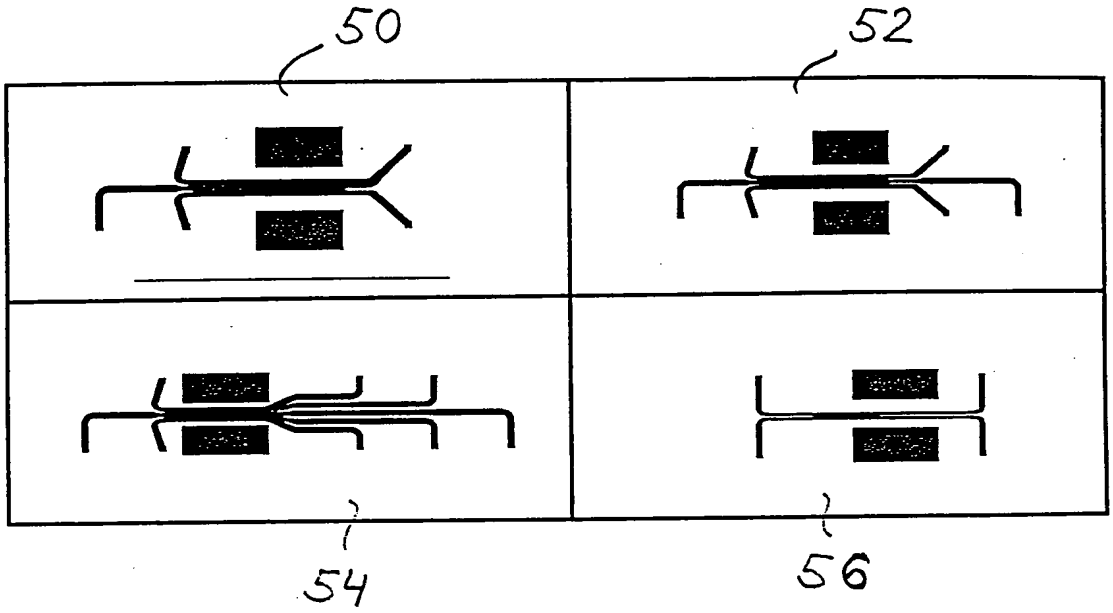

Fig. 4

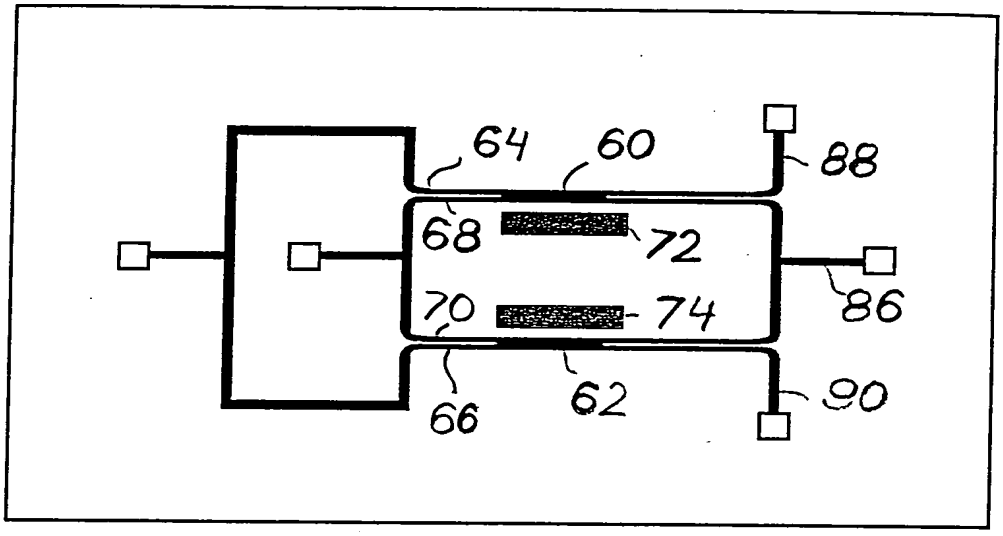

Fig. 5

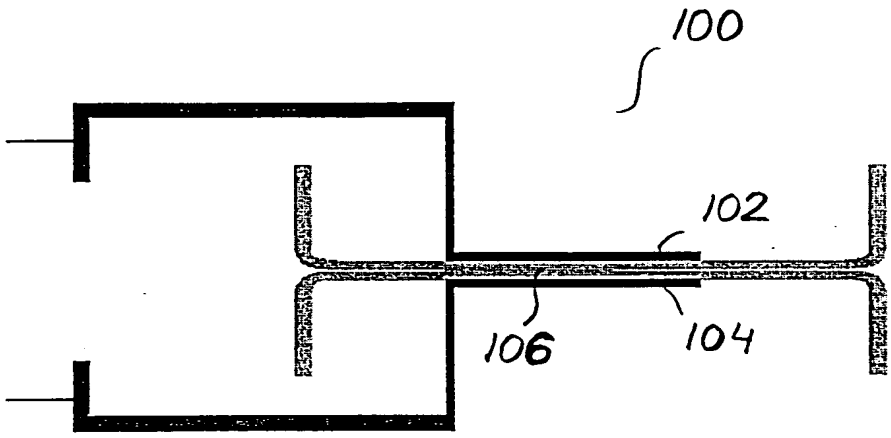

Fig. 6

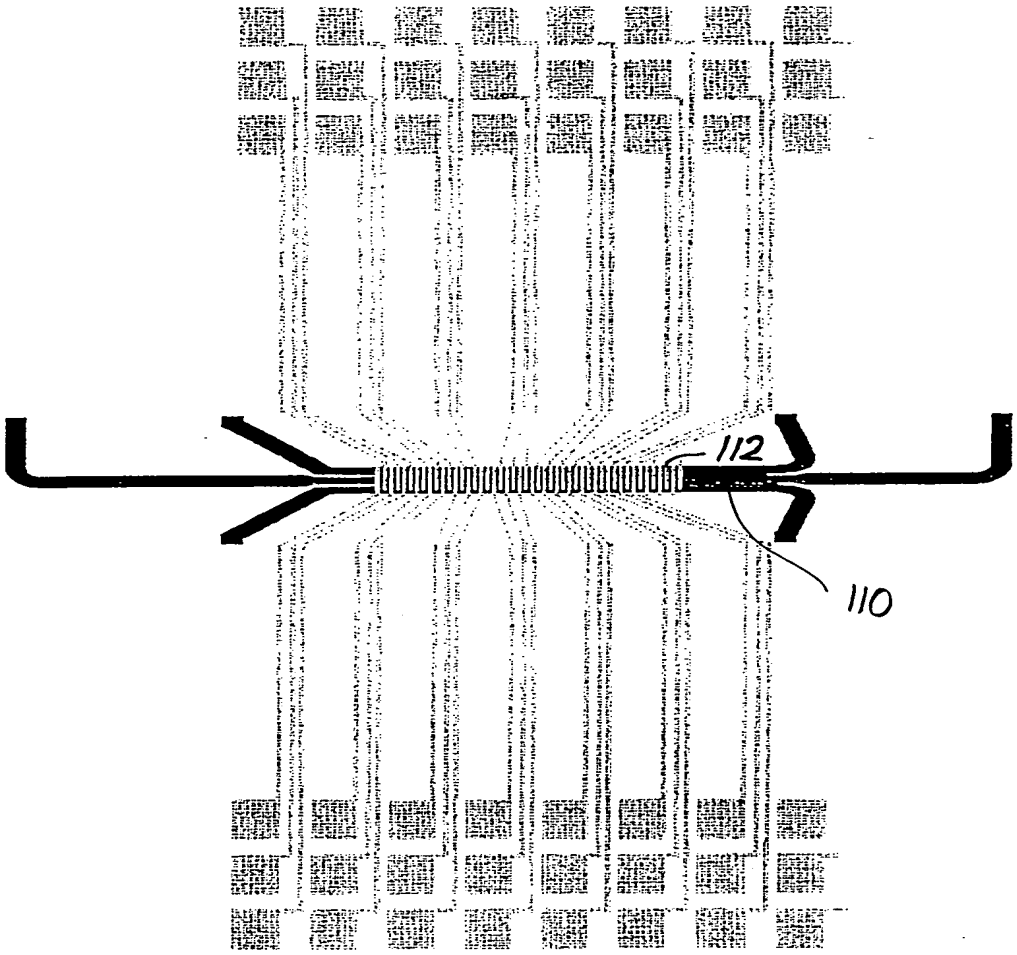

Fig. 7